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Characterization of the ModABC Molybdate Transport System of *Pseudomonas putida* in Nicotine Degradation

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Pseudomonas putida J5 is an efficient nicotine-degrading bacterial strain that catabolizes nicotine through the pyrrolidine pathway. In our previous study, we used Tn5 transposon mutagenesis to investigate nicotine metabolism-associated genes, and 18 nicotine degradation-deficient mutants were isolated from 16,324 Tn*5*-transformants. Three of the mutants were Tn5 inserts into the *modABC* gene cluster that encoded an ABC-type, high-affinity, molybdate transporter. In-frame deletion of the *modABC* genes abolished the nicotine-degrading ability of strain J5, and complementation with *modABC* either from *P. putida* or *Arthrobacter oxidans* restored the degrading activity of the mutant to wild-type level. Nicotine degradation of J5 was inhibited markedly by addition of tungstate, a specific antagonist of molybdate. Molybdate at a non-physiologically high concentration (100 μM) fully restored nicotine-degrading activity and recovered growth of the *modABC* mutant in a nicotine minimal medium. Transcriptional analysis revealed that the expression of *modABC* was up-regulated at low molybdate concentrations and down-regulated at high moybdate concentrations, which indicated that at least one other system was able to transport molybdate, but with lower affinity. These results suggested that the molybdate transport system was essential to nicotine metabolism in *P. putida* J5.

Keywords: biodegradation, nicotine, *Pseudomonas putida*, molybdate transporter, ModABC

INTRODUCTION

Molybdenum (Mo) plays essential roles in bacteria, because it serves as a cofactor for a number of enzymes that catalyze a variety of oxidation/reduction reactions, and it is involved in microbial metabolism of carbon, nitrogen, and sulfur (Hille, 1996; Kisker et al., 1997). For the synthesis of molybdoenzymes, bacteria need to transport molybdate, activate it to an appropriate form, and incorporate it into the organic part of the molybdenum cofactor (Zhang et al., 2011). In nature,